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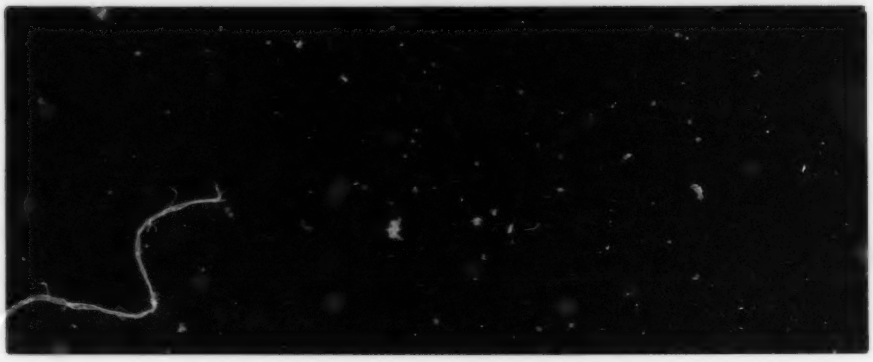

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Final Report

R-89-01-0438

Development of an Antibody-Based Staining Method for Diagnosis of Influenza A Virus in Pigs

Conducted by Dr. D. Haines and Dr. E. Clark

Funded by the Agriculture Development Fund

To: Saskatchewan Agriculture and Food Agriculture Development Fund Committee

From: Drs DM Haines and EG Clark, BIOdiagnosTEC, Western College of Veterinary Medicine

Re: Final Report Agriculture Development Fund Project No. R-89-01-0438

"Development of an antibody-based staining method for diagnosis of Influenza A virus in pigs"

Date: December 12, 1989

Technical Report

Abstract

Influenza A virus infection is known to be an important respiratory pathogen in pigs. The precise role of this agent in pig deaths due to respiratory disease is unknown since this micro-organism is extremely difficult to demonstrate by routine diagnostic techniques such as viral isolation. We have developed and verified the sensitivity and specificity of an antibody-based staining method for the detection of Influenza A virus in routinely formalin-fixed paraffin-embedded tissue sections. The method developed is convenient and rapid. The method was also shown to be sensitive, detecting 20/22 (91%) of cases positive by viral isolation, and specific, detecting 0/30 (0%) of those cases not thought clinically or histologically to be associated with Influenza infection. In this project we have successfully developed a staining method which can be used for the routine diagnosis of Influenza A infections and for retrospective and prospective studies of the occurrence of this virus in respiratory diseases in pigs.

Background and Objectives

Influenza A viruses cause respiratory diseases in many species including pigs, horses, poultry and man. The importance of this pathogen as a cause of death in pigs is difficult to assess since the routine diagnostic tests used to demonstrate the virus are inadequate. Attempts

to demonstrate Influenza A virus generally involve isolation of the virus in embryonated specific-pathogen free (SPF) eggs. This isolation method is extremely laborious, involves delays of up to two weeks for results, and in many cases which are suspected clinically and histologically to be due to Influenza infection, the isolation is negative which suggests that the test is possibly insensitive.

An improved technique for Influenza diagnosis could be achieved by immunohistochemical staining of affected tissues to demonstrate the viral antigens with antibodies that specifically bind to the virus. There are many commercially available antibodies to Influenza antigens designed primarily for detection of the virus in man. Since Influenza viruses infecting different species are antigenically similar, these commercially available antibodies could be used to diagnose infections in species other than man. In addition, immunohistochemical staining methods can be completed in a single day which is much more rapid than viral isolation which can take up to two weeks.

Immunohistochemical staining for disease diagnosis is especially convenient if applicable to formalin-fixed paraffin-embedded tissues. Formalin is the fixative used for routine histological examination of tissues, and the ability to demonstrate the virus in these fixed specimens means that a single sample may be used for both analysis. In addition these fixed specimens can be readily transported without special conditions. Another advantage of testing fixed tissues is that the time from sample collection to submission of the specimen to the laboratory is not critical since the organism is preserved by the fixative.

The project reported in this proposal is the development of an immunohistochemical staining method for the detection of Influenza A virus infections in porcine lungs using commercially available antibodies and formalin-fixed paraffin-embedded tissue specimens.

Experimental Methods

We first identified a number of commercially-available antibodies which were known to bind to Influenza A antigens. We chose antibodies which were directed against antigens which were likely to be common to the viruses affecting many species. These antibodies were tested in an

avidin-biotin complex (ABC) immunohistochemical staining method for the ability to detect the virus in a known-Influenza A infected tissue. One antibody was selected which showed brilliant staining of the nuclei of cells in known infected tissues and did not stain known uninfected tissues. Once the method was established, the sensitivity and specificity of the stain compared to virus isolation was tested.

To establish the sensitivity of the antibody-based staining method compared to virus isolation we have obtained tissues from 22 cases of swine respiratory disease in which the Influenza A virus was actually isolated. Since few suspect Influenza cases in Saskatchewan (or elsewhere) have actually been confirmed by virus isolation, most of these cases were obtained from laboratories in Ontario. Microscopic sections were cut from the paraffin blocks of tissues from cases in which Influenza A had been isolated and these were stained with the antisera to Influenza A antigens. Sections from the same blocks were also stained with an irrelevant antisera to test for non-specific staining of the sections.

To determine the specificity of the antibody-based staining test for influenza A antigens we have tested lung tissues from 30 cases in which the clinical symptoms of disease and the pathological examination of the tissues were not consistent with Influenza A disease (negative controls).

As a pilot test of the occurrence of Influenza A antigens in porcine respiratory disease we have also examined lung tissues from 22 cases of swine respiratory disease in which the clinical symptoms and/or histopathological examination suggested Influenza A infection, however the viral isolation culture techniques failed to detect the virus or viral isolation was not attempted.

The applicability of the test method to other domestic animal species was examined by testing known positive and negative tissues from horses and turkeys infected with Influenza A virus.

Results and Discussion

A commercially-available rabbit polyclonal antisera to whole influenza A virons was found to readily detect Influenza A viral antigens in known Influenza A infected pig lung specimens using an avidin-biotin

complex immunohistochemical staining technique on formalin-fixed paraffin embedded tissues. Positive staining was apparent as dark chocolate brown deposits primarily in the nuclei of epithelial cells of bronchi. Table 1 shows the results of staining of tissue sections of porcine lungs of 22 known positive Influenza A cases, 30 known negative cases and 22 cases in which either the clinical or histological disease was suggestive of Influenza A infection, however this was not confirmed by viral isolation.

In addition to these tests of pig tissues this staining method was tested on known positive and negative tissues from horses and from turkeys. In both of these species there was positive staining of cell nuclei in known Influenza A infected cases while known negative cases were unstained.

Table 1 Avidin-biotin complex immunohistochemical staining for Influenza A viral antigens in formalin fixed-paraffin-embedded tissue sections of cases in which Influenza A virus was isolated, in cases in which Influenza A was suspected by clinical or histological criteria but was not isolated and in cases in which Influenza A infection was not suspected.

CASES(NUMBER)	INFLUENZA A ANTIGEN STAINING	
	POSITIVE	NEGATIVE
INFLUENZA ISOLATED	19	2
INFLUENZA NOT SUSPECTED	0	30
INFLUENZA SUSPECTED*	5	17

* cases in which the clinical or histological disease was suggestive of Influenza A however this was not confirmed by viral isolation.

Conclusions and Impact

The assay developed in this study is a convenient, sensitive and specific means of diagnosing Influenza A infections in pigs which die or are sacrificed with respiratory disease. This is a particularly valuable test in Saskatchewan and the other prairie provinces in which the producers are often large distances from the diagnostic service laboratory, since the specimens used in this test can be readily fixed in formalin just as for routine histological examination, then easily transported without time constraints. Also of importance is that this test enables retrospective diagnosis, which can be useful in instances in which Influenza A infection is not suspected clinically, however after histological examination the disease is suspected. Fresh tissue for viral isolation will not be available in these cases, however with the staining method developed in this study the virus can readily be demonstrated in the majority of cases. The finding of 5/22 positive Influenza A cases in pigs dying of respiratory disease with either clinical or histological evidence suggestive of this disease demonstrates that this method could be used for a study of the occurrence of this virus in porcine respiratory disease.

Once the tissues are embedded in paraffin the specimens may be retrospectively examined at any time. Some of the specimens tested in this study were over 10 years old. The ability to test stored specimens enables large scale retrospective examinations to determine the incidence and importance of this pathogen in respiratory deaths in pigs. Since the tissues stained for the viral antigen can be simultaneously counterstained with the routine fixatives used in histological examination, the morphological detail of the tissues can be readily examined along with the distribution of the virus to assess the role of the virus in the pathogenesis of the lesions.

This test may be particularly valuable to rapidly diagnose Influenza A infection in minimal disease pig herds, where deaths may not occur but one or two pigs are sacrificed during an obvious outbreak of respiratory disease. These minimal disease herds are often suppliers of stock and confirmation of such a disease in the population is especially important to these producers.

Papers and Talks

Preliminary results of this study were discussed in a presentation (Dr Haines) to the Western Canadian Meeting of Swine Practitioners, Oct 5, 1989 in Saskatoon. A manuscript describing these findings is in preparation and will be submitted to a veterinary journal for consideration for publication. In addition the stained tissues will be utilised by a graduate student in Veterinary Pathology at WCVM (Dr E Waters) to describe the relationship between distribution of Influenza A virus antigens and histological lesions. This will constitute a Masters of Veterinary Science study and will be presented as a seminar at WCVM and will be submitted for consideration for publication in a veterinary journal.

Statement of Expenditures

An account of expenditures will be forwarded directly from the University of Saskatchewans' Office of Research Accounts.

Personnel Involved

In addition to the principle investigators, personnel involved in this study is Mr. Brian Chelack a laboratory technologist. Mr Chelack has devoted about 1/10-1/20 of his time to this study over a 4 month period.

Equipment Purchased

No equipment was purchased with funds from this study.

Executive Summary

Abstract

Influenza A virus is an important cause of pneumonia in pigs. The accurate diagnosis of disease due to this virus has been problematic because the virus is extremely difficult to isolate. We have developed an improved technique for diagnosing Influenza A virus infection in pigs. The method is a staining technique on tissue specimens using antibodies which are sold commercially for detection of Influenza A infections in people. Some of these antibodies were found to cross-react to bind to Influenza antigens in pigs. This antibody-based staining method was tested on tissues collected from pigs known to be infected with Influenza A virus and on tissues from pigs not infected with this virus. The staining method developed in this study was positive in 19/22 cases (91%) of known positive cases and in none of 30 known negative cases. The method developed in this study is a sensitive and specific method for demonstration of Influenza A virus infection in pigs and will enable accurate and rapid diagnosis of this pathogen.

Summary

In this project we have developed an improved method for the diagnosis of Influenza A virus infection in pigs. The method developed relies on the detection of the virus in tissue specimens from affected pigs using antibodies specific for the virus. The binding of the antibodies to the virus in the tissues is detected by an enzyme-based staining technique which makes the binding of the antibody microscopically visible. This staining method can be completed in less than one day compared with attempts to isolate the virus which can take up to two weeks.

We have tested a number of antibodies which are sold commercially for detection of Influenza A infections in people. Some of these antibodies were found to cross-react to bind to Influenza antigens in pigs. We have obtained 22 cases of swine respiratory disease in which the Influenza A virus was actually isolated. In 20/22 (91%) of these cases the staining method developed in this study was also positive. We have also tested tissues from 30 cases of pig respiratory disease which were not suspected to be associated with Influenza A and in 30/30 (100%) of these cases there was no of staining of the tissues with this method.

The assay developed in this study is a convenient, sensitive and specific means of diagnosing Influenza A infections in pigs which die or are sacrificed with respiratory disease. This is a particularly valuable test in Saskatchewan and the other prairie provinces in which the producers are often large distances from the diagnostic service laboratory, since the specimens used in this test can be readily fixed in formalin just as for routine histological examination, then easily transported without time constraints.

Also of importance is that this test enables retrospective diagnosis, which can be useful in instances in which Influenza A infection is not suspected clinically, however after histological examination the disease is suspected. Fresh tissue for viral isolation will not be available in these cases, however with the staining method developed in this study the virus can readily be demonstrated in the majority of cases.

This test may be particularly valuable to rapidly diagnose Influenza A infection in minimal disease pig herds, where deaths may not occur but one or two pigs are sacrificed during an obvious outbreak of respiratory disease. These minimal disease herds are often suppliers of stock and confirmation of a disease in the population is especially important to these producers.